Figure 1. Representative FACS profiles of the isolation of CD271$^{+/-}$SSEA-4$^{+/-}$ subpopulations from Lin^{-}CD45^{-} human bone marrow (BM) cells.

(A) The immunomagnetically separated lineage-negative cells were gated by forward scatter (FSC) and side scatter (SSC: R1). (B) Dead cells were excluded by 7-AAD staining (R2). (C) The eleven lineage (CD2, CD3, CD4, CD14, CD16, CD19, CD24, CD41, CD56, CD66c and CD235a)-negative (R3) and (D) CD45-negative fraction (R4) was gated. R4-gated cells were further subdivided into four fractions according to their expressions of the CD271 and SSEA-4 antigens (R5-R8). The percentages of these subdivided Lin^{-}CD45^{-} fractions were 3.3-42.5% (SSEA-4 SP, R5), <0.1-3.1% (DP, R6), 50.7-93.4% (DN, R7) and <0.1-6.8% (CD271 SP, R8), respectively. The presented FACS plot shows representative data from seven independent biological replicates.

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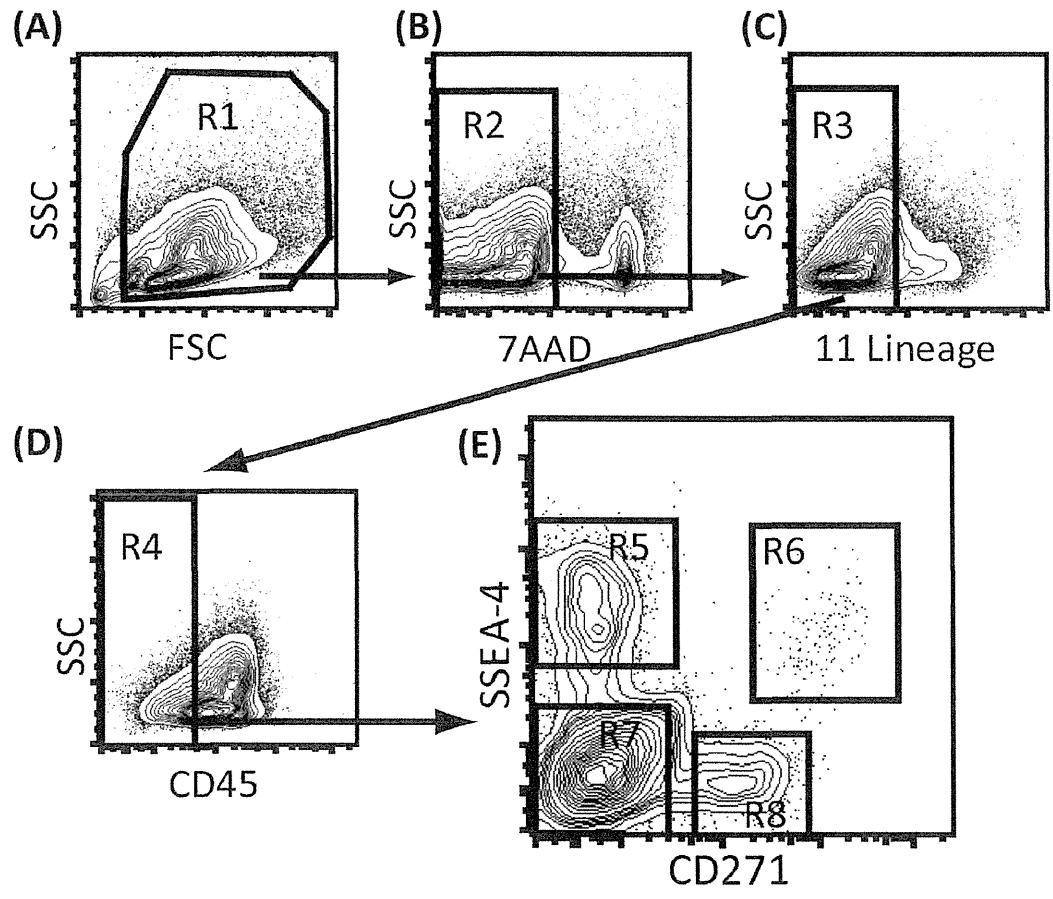

Figure 2. Colony-forming unit-fibroblast (CFU-F) capacities and *in vitro* cellular characteristics of the subdivided BM-derived Lin⁻CD45⁻ cells.

(A-D) The representative images of CFU-F colonies from each BM fraction. CFU-Fs formed from (A) CD271⁻SSEA-4⁺ (SSEA-4 SP), (B) CD271⁺SSEA-4⁺ (DP), (C) CD271⁻SSEA-4⁻ (DN) and (D) CD271⁺SSEA-4⁻ (CD271 SP) fractions were stained with May-Grunwald-Giemsa (MG) after 13 days in culture. The mean frequencies of CFU-Fs from the two independent experiments (n = 6) are indicated in the upper corner of each image. Scale bar: 3 cm. (E-G) Phase contrast images of cultured MSCs at the passage four are shown. Representative images of MSCs established from (E) DP, (F) DN and (G) CD271 SP cells. Scale bar: 250 μm. (H) The forward scatter histograms of MSCs established from DP (black line), CD271⁺ SP (dotted line) and DN (gray filled line) MSCs. The values of the mean fluorescence intensity (MFI) of the FSC of each MSC are indicated above the histograms. (I) The growth curves of the MSCs established from subdivided BM Lin⁻CD45⁻ cells. Each line is depicted in the figure. The photographs were recorded by using the FinePix S5 Pro device (Fujifilm, Tokyo, Japan) and the Studio Utility (version 1.0.2.3.) software program (Fujifilm).

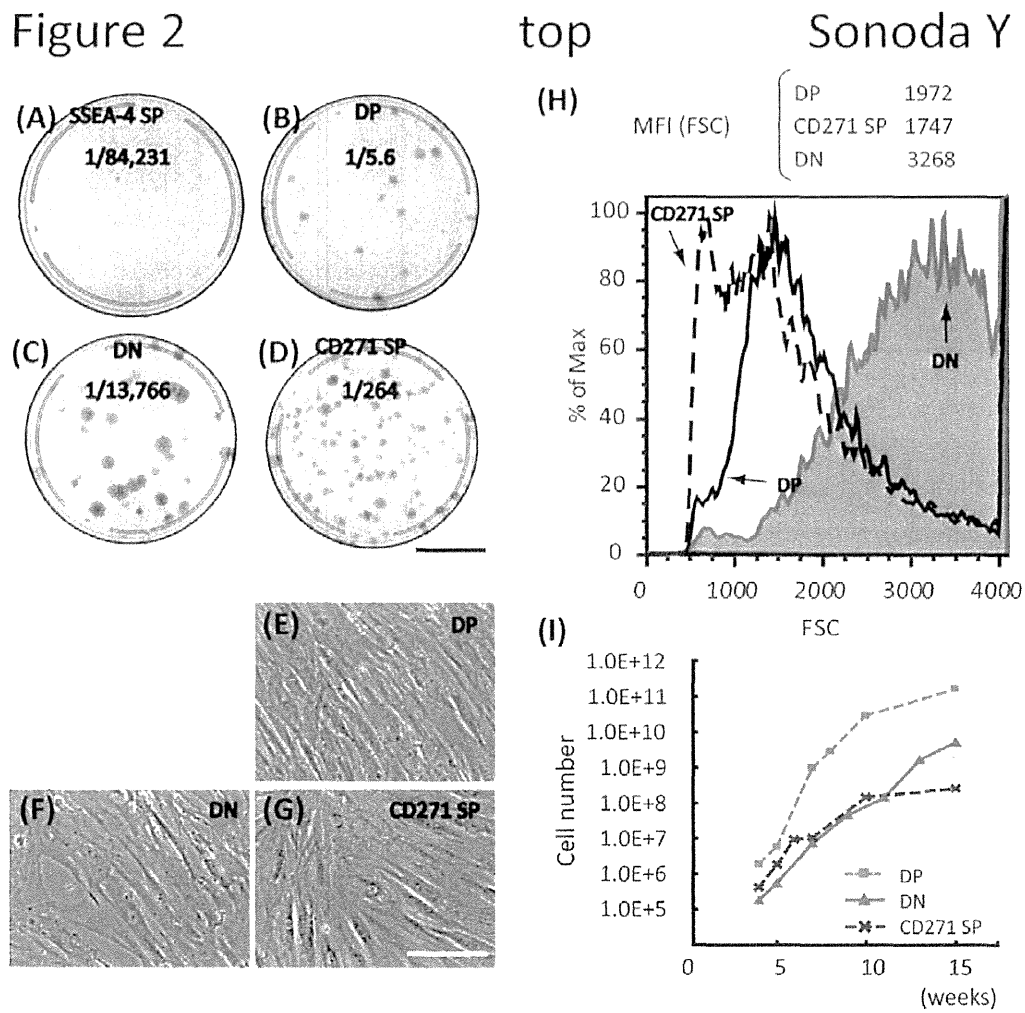

Figure 3. The surface marker expression of each type of BM-MSCs.
(A) At the passage 4, the cultured MSCs were collected and stained with monoclonal antibodies against MSC, hematopoietic cell, and endothelial cell markers. The gray filled line and black line represent specific and control isotype antibody staining, respectively. (B) The expression of CD271 was downregulated during the passaging of the DP MSCs. The left, center, and right panels show the expression of CD271 in the DP MSCs at the passage (P) 0, 1, and 2, respectively.

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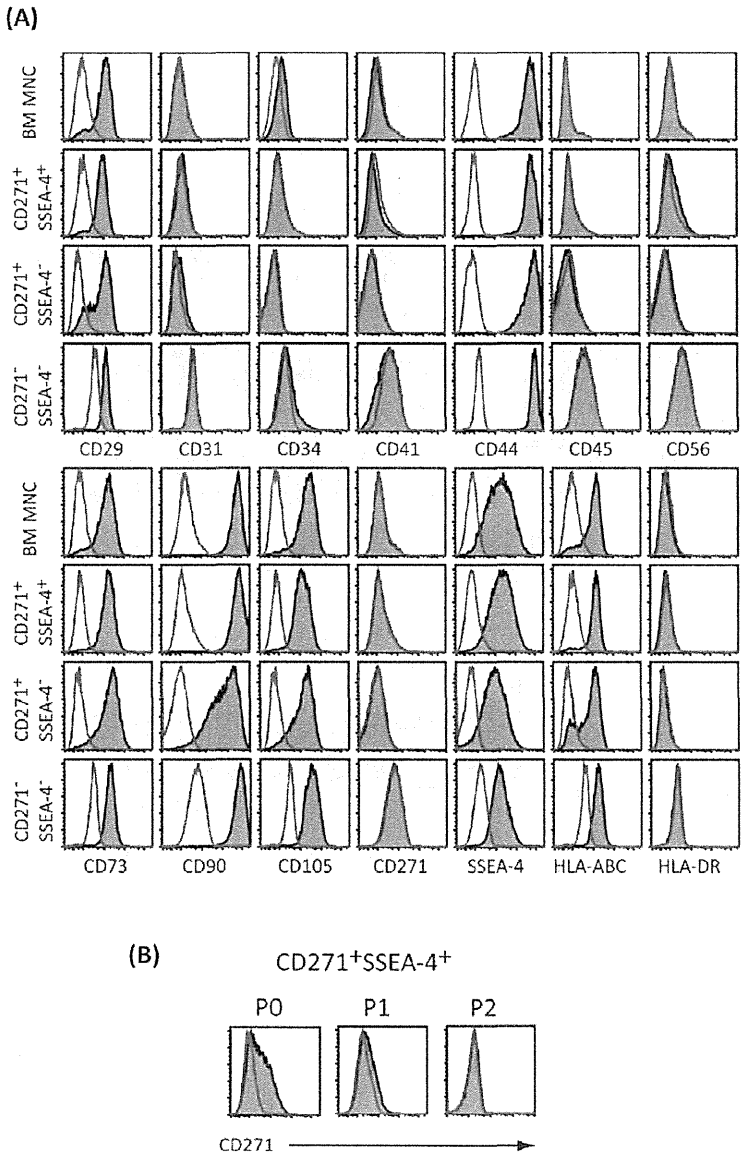

Figure 4. The osteogenic, adipogenic and chondrogenic differentiation capacities of BM-MSCs.
The MSCs were cultured under appropriate conditions to induce their differentiation into osteoblasts, adipocytes and chondrocytes for 21 days. Cells were fixed and counter-stained with Alizarin Red S and Oil Red O, and were immunohistochemically stained for osteocalcin, FABP-4, and aggrecan. Green: osteocalcin, Red: Alizarin Red S, Oil Red O, FABP4 or aggrecan, as indicated. Blue: nuclei (Hoechst 33342-stained). No adipogenic differentiation was observed in the DP MSC culture (highlighted by red square). Scale bar: 250 μm.

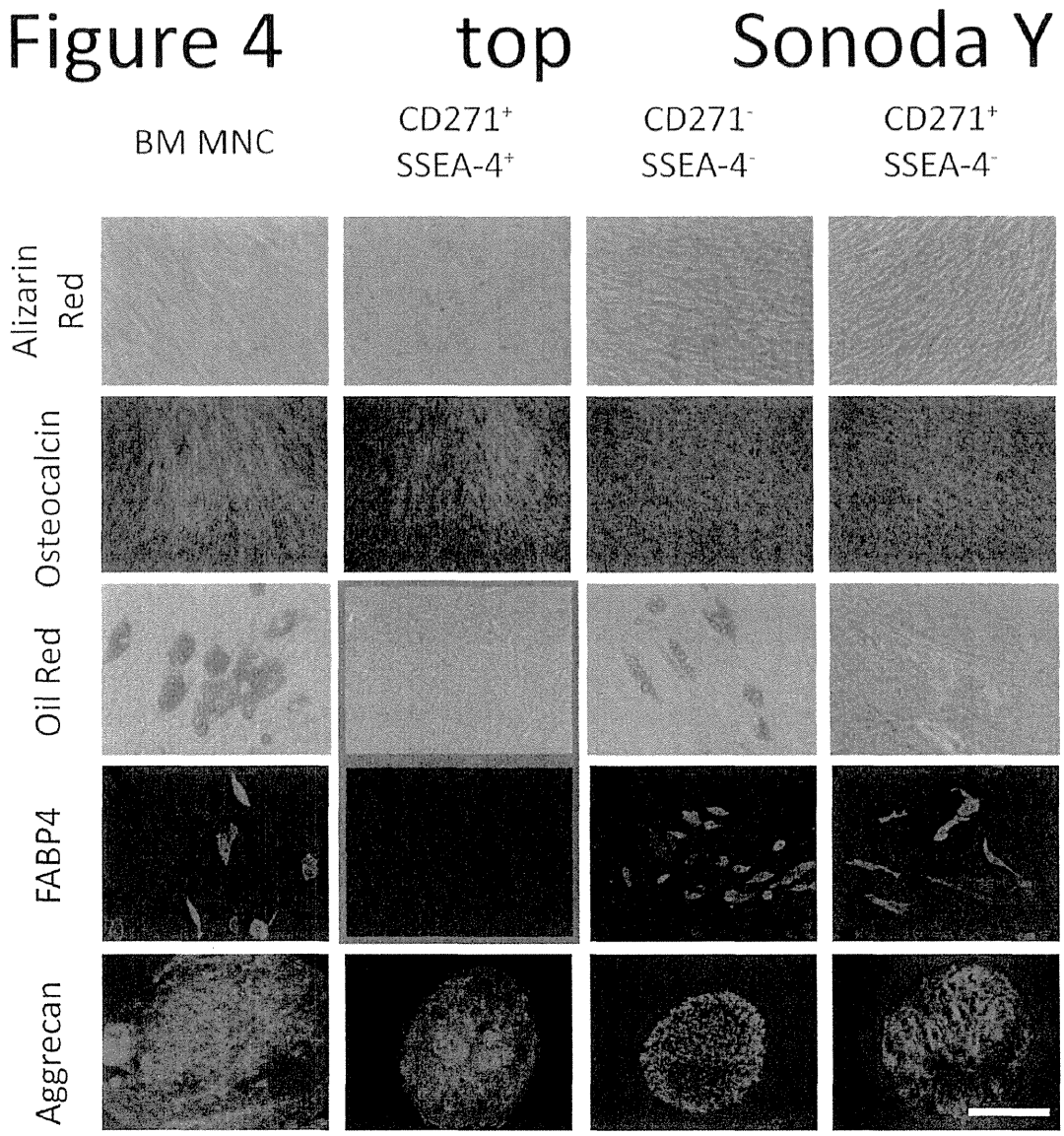

Figure 5. The expressions of osteogenic, adipogenic, and chondrogenic differentiation genes, MSC marker genes and hematopoietic stem cell-supportive genes in established BM- MSCs.
The gene expression levels of established BM-derived MSCs were estimated by qRT-PCR. The gene expression levels were normalized by GAPDH. The relative expression levels of each gene were compared with those of the unfractionated BM MNC-derived MSCs. The data are presented as the mean values ± SD from three independent experiments. *P < 0.05, **P < 0.01

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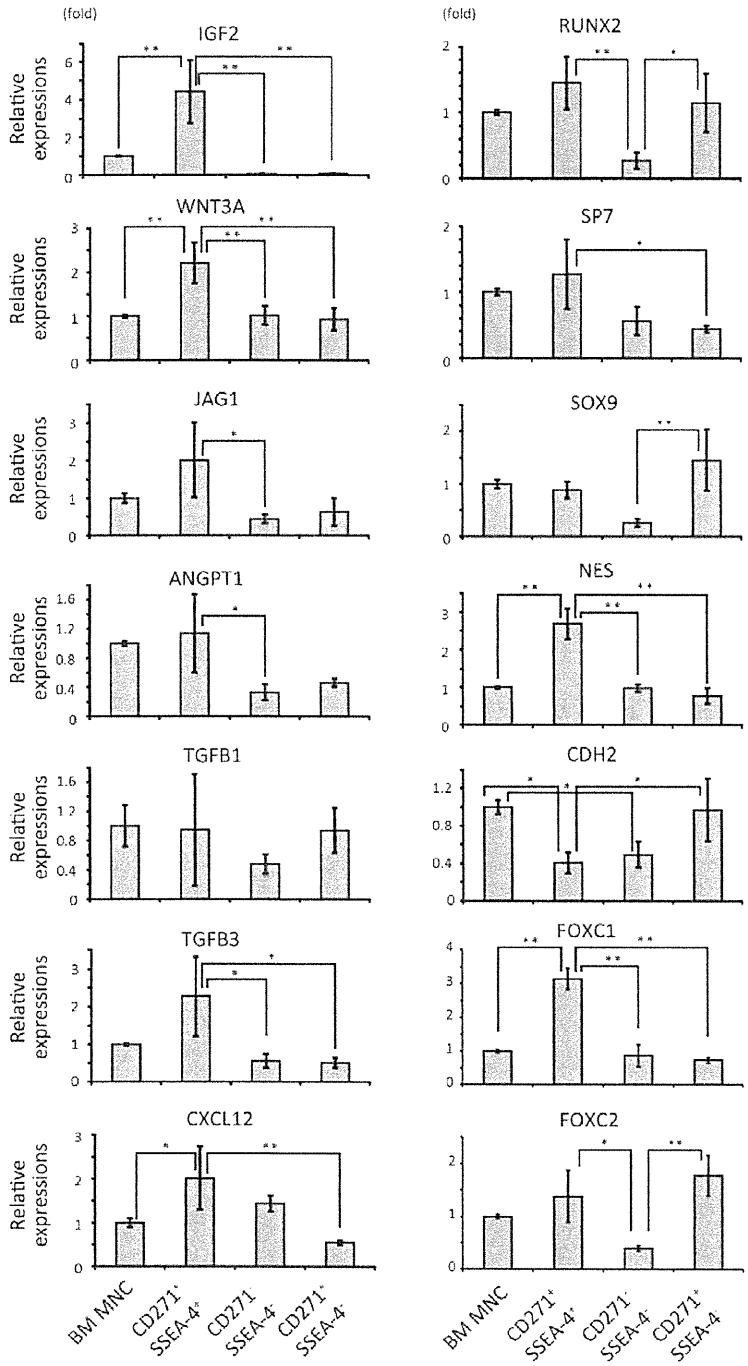

Figure 6. The SCID-repopulating cell (SRC)-supportive abilities of subdivided BM-MSCs *in vitro*.
CB-derived 18Lin⁻CD34⁻ cells were cultured with or without established MSCs in the presence of a cocktail of cytokines. After seven days, the cells were collected and transplanted into NOG mice by IBMI. The human CD45⁺ cell rates in the left tibia (injected site), right tibia and both the femur (other bone) and peripheral blood of NOG mice were analyzed 20 weeks after transplantation. The horizontal bar indicates the mean level of human CD45⁺ cells. Each dot represents the human cell engraftment of individual mice. *P < 0.05, **P < 0.01.

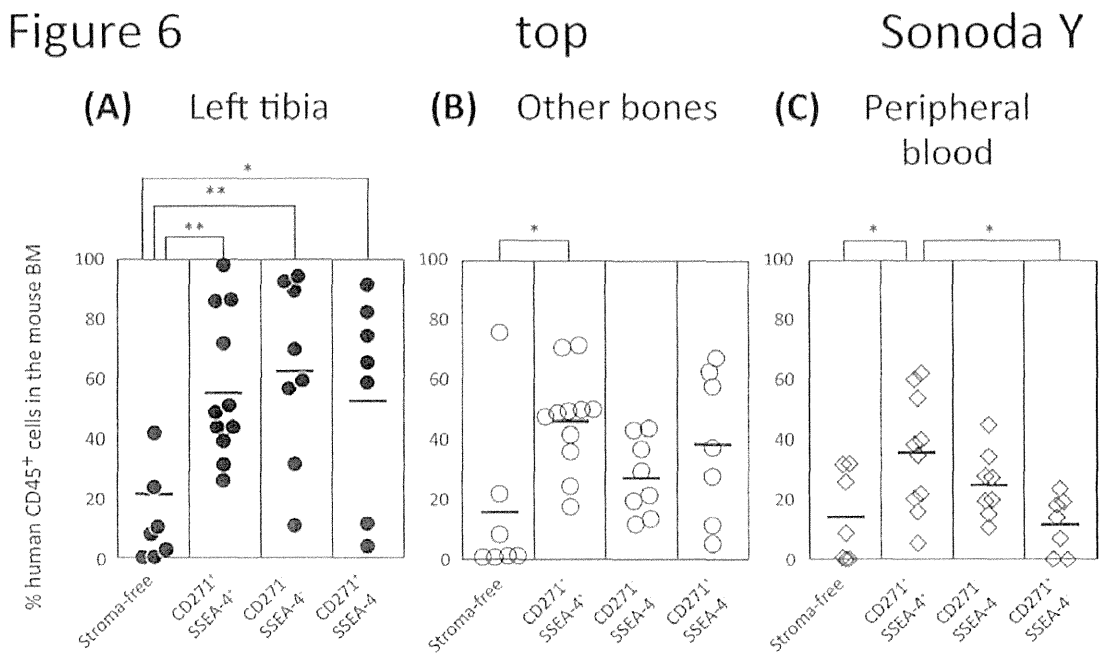

Figure 7. The growth, differentiation capacities, surface marker expressions and 18Lin$^-$CD34$^{+/-}$ cell supportive abilities of DP MSCs established from single CD271$^+$SSEA-4$^+$ cells.

(A) Single BM Lin$^-$CD45$^-$CD271$^+$SSEA-4$^+$ cells were sorted onto the 96-well-plates. The CFU-F colonies were assessed at day 13. The results of two independent experiments are shown. (B) The total numbers of cells derived from single cell-derived DP MSCs on day 40. The black bar indicates the median value of the cell number. (C) The osteogenic and adipogenic differentiation capacities of three single cell-derived DP MSCs are shown. Cells were induced to differentiate, and were visualized as shown in Figure 4. Scale bar: 250 μm. (D) The surface marker expression of six clonally cultured DP MSCs was analyzed on day 40. Cells were dissociated from the culture plates and reacted with mAbs as indicated in Figure 3. (E) 18Lin$^-$CD34$^+$ (1.5 x 10^3 cells per well) and CD34$^-$ cells (3.0 x 10^3 cells per well) were cultured on the feeders of the three DP MSC clones. After one week of coculture, the cells were collected, and the number of cells and percentages of CD34$^+$ cells were assessed. The fold- increases were calculated based on the number of cells collected and the number of cells input initially. The percentages of CD34$^+$ cells were analyzed by FCM.

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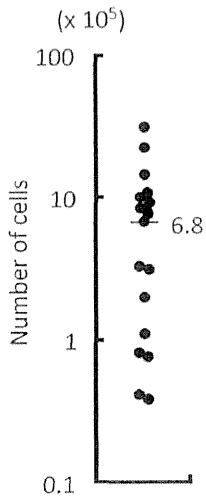
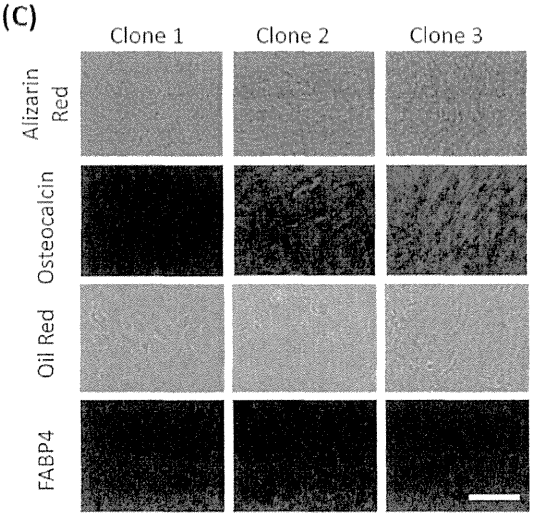
(A)

	Positive well	Frequency
Experiment 1	15/96	1/6.4
Experiment 2	18/96	1/5.3

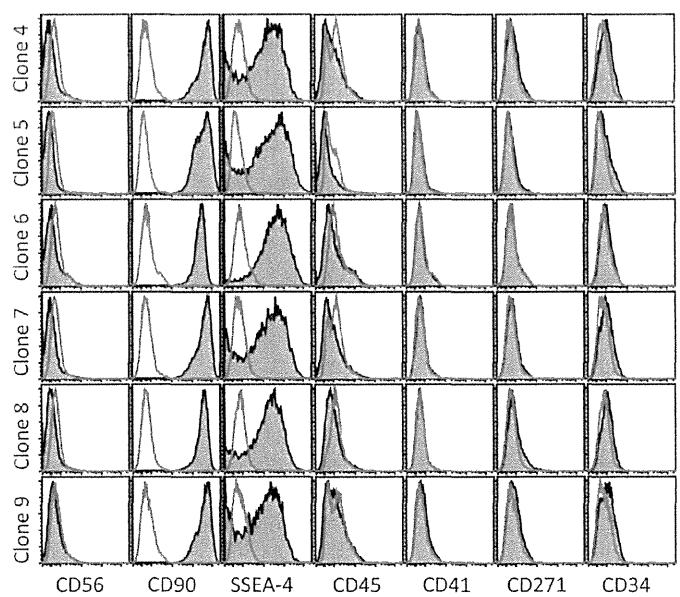
(B)



(C)



(D)



(E)

	Fold increase		% of CD34⁺ cell	
	CD34⁺	CD34⁻	CD34⁺	CD34⁻
Clone 6	106	67	24.3	25.6
Clone 7	116	73	7.5	11.0
Clone 8	146	89	24.9	26.5

bjh research paper

Haematopoietic stem cell transplantation for relapsed or refractory anaplastic large cell lymphoma: a study of children and adolescents in Japan

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Received 30 June 2014; accepted for publication 4 September 2014

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Summary

To evaluate haematopoietic stem cell transplantation (HSCT) in children and adolescents, we reviewed the records of 47 patients who were ≤18 years, had relapsed or refractory anaplastic large cell lymphoma, and received HSCT between 1990 and 2010. At HSCT, complete remission (CR) was less common in allogeneic HSCT recipients ($n = 24$) than in autologous HSCT recipients ($n = 23$) ($P = 0{\cdot}01$). The autologous and allogeneic HSCT groups differed in terms of 5-year event-free survival (EFS) (38% vs. 50%, $P = 0{\cdot}63$), cumulative incidence of progress or relapse (49% vs. 28%, $P = 0{\cdot}25$), and treatment-related mortality (12% vs. 25%, $P = 0{\cdot}40$). However, these differences were not significant. Patients with non-CR at autologous HSCT had a significantly lower EFS rate (14% vs. 48%, $P = 0{\cdot}03$). Conversely, although those with non-CR at allogeneic HSCT had a lower EFS rate, this was not significant (44% vs. 63%, $P = 0{\cdot}26$). Reduced-intensity conditioning regimens were used for three of the 16 allogeneic HSCTs received by patients with non-CR. These three patients achieved CR, surviving 32–65 months after HSCT. These results demonstrated that allogeneic HSCT might be a treatment option for patients who do not achieve CR through conventional chemotherapy.

Keywords: anaplastic large cell lymphoma, children, adolescents, haematopoietic stem cell transplantation, reduced-intensity conditioning.

First published online 14 October 2014
doi:10.1111/bjh.13167

Anaplastic large cell lymphoma (ALCL) is rare in children, accounting for 10–15% of childhood non-Hodgkin lymphoma cases (Murphy, 1994). The event-free survival (EFS) rate is 65–75% in children and adolescents receiving a first-line strategy based on short-pulse chemotherapy over a period of 3–6 months (Brugières *et al*, 1998, 2009a; Seidemann *et al*, 2001; Le Deley *et al*, 2010). Accordingly, the relapse rate is approximately 30% in most study series. The treatment of relapsed and refractory ALCL remains a matter of debate. Patients with relapsed ALCL have a 30–60% chance of survival under current treatment strategies, which include high-dose chemotherapy with haematopoietic stem cell transplantation (HSCT) and long-term treatment with vinblastine (Brugières *et al*, 2000, 2009b; Williams *et al*, 2002; Mori *et al*, 2006; Woessmann *et al*, 2006; Stockklausner *et al*, 2008; Gross *et al*, 2010). In contrast, patients who experience ALCL progression during first-line chemotherapy have extremely poor outcomes (Woessmann *et al*, 2006) and autologous or allogeneic HSCT is required as the most appropriate therapy.

Some evidence is available regarding the roles of autologous and allogeneic HSCT in paediatric ALCL. However, data are limited to several HSCT case series and case reports. In particular, few reports have been published regarding allogeneic HSCT for paediatric ALCL. We previously reported a retrospective analysis of 26 paediatric patients with recurrent ALCL in Japan (Mori *et al*, 2006). In that study, only three of the eight patients who received autologous HSCT while in their second complete remission (CR) survived without further relapse. In contrast, all six patients who received allogeneic HSCT while in their second CR survived without further relapse. However, our previous study included too few patients for us to discuss the efficacy of HSCT for relapsed or refractory childhood ALCL.

In the present study, we sought to evaluate the efficacy of HSCT for relapsed or refractory ALCL in children and adolescents. We performed a further retrospective analysis of 47 patients who received autologous or allogeneic HSCT for relapsed or refractory ALCL between 1990 and 2010.

Patients and methods

Patients and transplantations

This study was approved by the institutional ethics committee of National Kyushu Cancer Centre. Data on patients who had undergone HSCT were collected from the registries belonging to the Transplant Registry Unified Management Program system of the Japan Society for Hematopoietic Cell Transplantation. The study included 47 patients who had a diagnosis of relapsed or refractory ALCL and received HSCT at age ≤18 years between March 1990 and September 2010. Twenty-three patients received autologous HSCT and 24 patients received allogeneic HSCT. Refractory disease was defined as progression during fist-line treatment. Reduced-intensity conditioning (RIC) regimens were defined as (a) total body irradiation of ≤500 cGy as a single fraction or ≤800 cGy if fractionated, (b) <9 mg/kg of busulfan, (c) ≤180 mg/m^2 of melphalan, (d) <10 mg/kg of thiotepa, or (e) the BEAM regimen (carmustine, etoposide, cytarabine and melphalan), according to previous reports (Yaniv & Stein, 2008; Giralt *et al*, 2009; Ohta *et al*, 2010; Luger *et al*, 2012). All other conditioning regimens were defined as myeloablative conditioning (MAC) regimens.

Statistical analysis

Overall survival (OS), EFS, cumulative incidences of relapse and treatment-related mortality (TRM) were estimated using the Kaplan Meier method. The Mann–Whitney *U* test, χ^2-test, and Fisher's exact test were used to assess differences in patient characteristics. The level of statistical significance was set at $P < 0{\cdot}05$. All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Autologous HSCT

The patients' characteristics are shown in Table I. Twenty-three patients received autologous HSCT for relapsed or refractory disease as their first transplantation. The median follow-up duration for survivors after autologous HSCT was 154 (range: 9–224) months. The median age at HSCT was 15 (range: 7–18) years. Sixteen patients had achieved CR at HSCT and seven patients had residual disease. Bone marrow and peripheral blood were the stem cell sources in three and 20 patients, respectively. Engraftment was observed in 23 (100%) cases, occurring at a median of 12 d. The 5-year cumulative incidence of relapse was 49% ± 11% (Fig 1A). Treatment-related death occurred in three of the patients who received autologous HSCT and the 5-year cumulative incidence of TRM was 12% ± 9% (Fig 1B). Two of the three patients died of infectious complications and one patient died of multiple organ failure. The 5-year OS and EFS rates were 51% ± 11% and 38% ± 10%, respectively (Fig 2A, B). We observed 5-year EFS rates of 48% ± 13% and 14% ± 13% for patients with CR and non-CR, respectively, at autologous HSCT (Fig 3A), which constituted a significant difference ($P = 0{\cdot}03$).

Allogeneic HSCT

Twenty-four patients received allogeneic HSCT for relapsed or refractory disease (Table I). The median follow-up duration for survivors after allogeneic HSCT was 68 (range: 32–212) months. The median age at HSCT was 13·5 (range: 3–18) years. Of the 24 patients, four had received previous autologous HSCT. Eight patients had achieved CR at HSCT and 16 patients had residual disease (Table I). The sources of stem cells were bone marrow in 13 patients, cord blood in

Table I. Characteristics of patients with relapsed or refractory ALCL according to the receipt of autologous or allogeneic HSCT.

	Autologous	Allogeneic	*P*
Patients (*n*)	23	24	
Age at HSCT (years)			
Median	15	13·5	0·27
Range	7–18	3–18	
Sex			
Male	17	21	0·24
Female	6	3	
Stage at diagnosis			
I	1	0	0·36
II	3	4	
III	11	6	
IV	4	8	
Unknown	4	6	
Disease status at HSCT			
CR2/CR≥3	14/2	5/3	0·01
Non-CR	7	16	
Conditioning			
TBI/TLI based	7/1	17/1	0·06
Non-TBI based	15	6	
Stem cell source			
BM	3	13	
PB	20	5	
CB	0	6	
Donor			
MRD	–	7	
MUD	–	2	
MMRD	–	6	
MMUD	–	7	
Unknown	–	2	

HSCT, haematopoietic stem cell transplantation; CR, complete remission; BM, bone marrow; CB, cord blood; PB, peripheral blood; MRD, matched related donor; MUD, matched unrelated donor; MMRD, mismatched related donor; MMUD, mismatched unrelated donor; TBI, total body irradiation; TLI, total lymphoid irradiation.

six patients and peripheral blood in five patients. Seven patients had human leucocyte antigen (HLA)-matched related donors, and two patients received stem cells from HLA-matched unrelated donors. Thirteen patients had HLA-mismatched donors. Engraftment was observed in 21 (88%) cases, occurring at a median of 17 d. Two patients died of infection and one died of disease progression before engraftment. The 5-year cumulative incidence of relapse was 28% ± 10% (Fig 1A). Treatment-related death occurred in five patients; four patients died of infectious complications and one patient died of acute graft-*versus*-host disease (GVHD). The 5-year cumulative incidence of TRM was 25% ± 10% (Fig 1B). Acute GVHD of any grade occurred in 13 patients, nine of whom had grade II–IV GVHD. The 5-year OS and EFS rates were 54% ± 10% and 50% ± 10%, respectively (Fig 2A, B). Seven of 24 patients had multiple relapses before their HSCT; the 5-year EFS rates among patients with and without multiple relapses were 43% ± 19% and 53% ± 12%, respectively ($P = 0·67$). We observed 5-year EFS rates of 63% ± 17% and 44% ± 12% among patients with CR and those with non-CR respectively, at allogeneic HSCT (Fig 3B), which did not constitute a significant difference ($P = 0·13$).

At HSCT, CR was less common among allogeneic HSCT recipients than it was among autologous HSCT recipients ($P = 0·01$). However, there were no significant differences between the autologous and allogeneic HSCT patients in terms of cumulative incidence of relapse ($P = 0·25$), cumulative incidence of TRM ($P = 0·40$), 5-year OS ($P = 0·95$) or 5-year EFS ($P = 0·63$).

RIC regimens

Of the 24 patients in the allogeneic group, four underwent allogeneic HSCT using RIC. Their outcomes are shown in Table II. One of the four patients died of bacterial infection and the other three patients survived in CR without relapse after allogeneic HSCT. Interestingly, none of these three patients were in CR at HSCT.

Discussion

Currently, the efficacy and toxicity of HSCT are poorly defined for childhood cases of relapsed or refractory ALCL. Evidence is especially lacking in regards to the efficacy and toxicity of allogeneic HSCT. The present study included 23 patients who underwent autologous HSCT and 24 patients who underwent allogeneic HSCT. Each of the patients was a child or adolescent who had relapsed or refractory ALCL and underwent HSCT in Japan. This report comprises the largest cohort concerning allogeneic HSCT for relapsed or refractory ALCL in childhood.

The Berlin-Frankfürt-Münster (BFM) cohort had efficacies of autologous HSCT (77% OS and 59% EFS among 39 children with relapsed ALCL) that lie at or above the upper range of previously reported series (Woessmann *et al*, 2011). In national case series from the United Kingdom and France, one of six and nine of 15 patients stayed in continuous CR (Brugières *et al*, 2000; Williams *et al*, 2002; Woessmann *et al*, 2011). The Center for International Blood and Marrow Transplant Research (CIBMTR) has reported another large series of autologous HSCTs that were performed for ALCL, noting an EFS of 35% in 24 patients (Gross *et al*, 2010). Previously, we have reported a retrospective analysis of relapsed ALCL, which included 26 patients in Japan (Mori *et al*, 2006). Three of the eight patients who underwent autologous HSCT survived in continuous CR. In the current study, the 5-year OS rate, EFS rate and cumulative incidence of relapse among the 23 patients who underwent autologous HSCT were 51%, 38% and 49%, respectively. These results are similar to the findings of a previous CIBMTR report (Gross *et al*, 2010). In a study of 64 adult and paediatric cases of autologous HSCT for ALCL, Fanin *et al* (1999) reported that disease status at HSCT

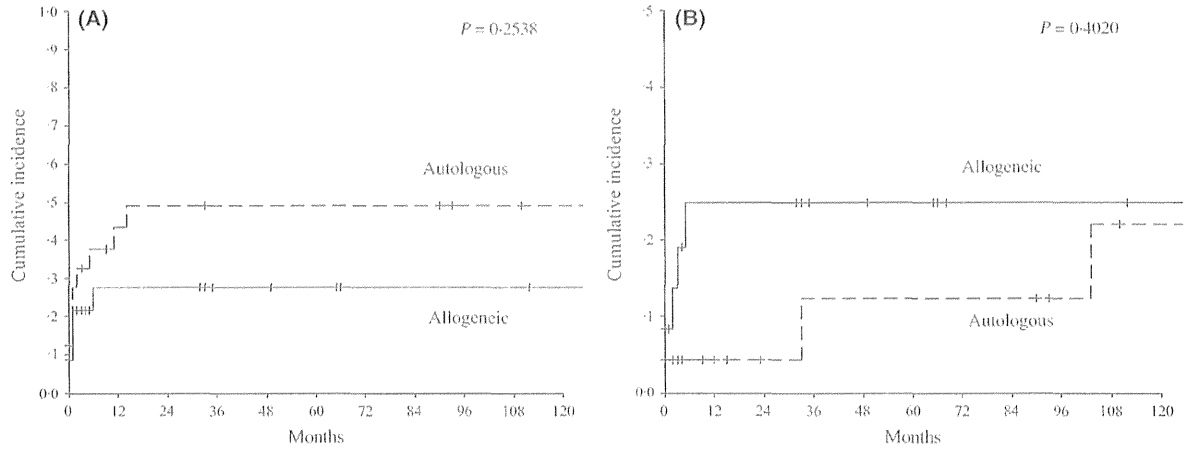

Fig 1. The cumulative incidence of relapse (A) and treatment-related mortality (B) according to autologous and allogeneic haematopoietic stem cell transplantation.

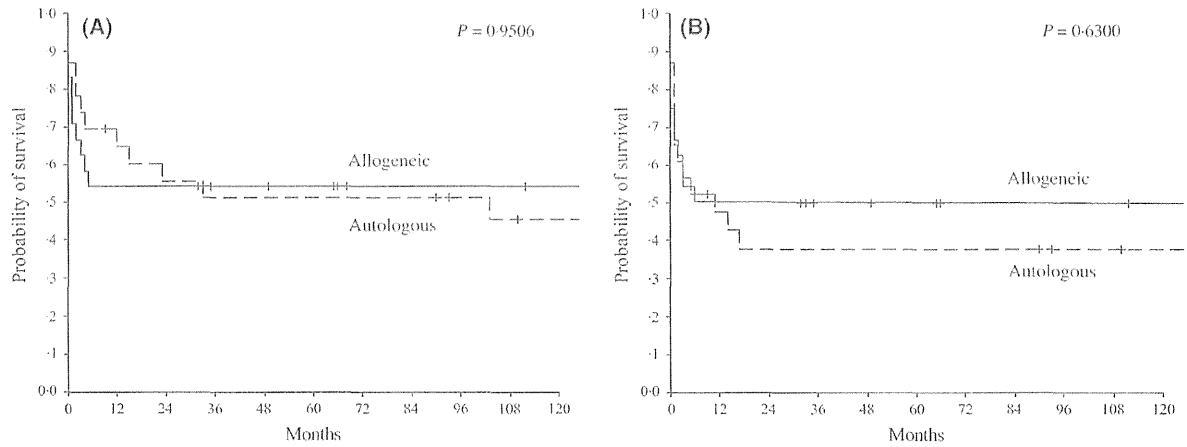

Fig 2. Overall survival (A) and event-free survival (B) according to autologous and allogeneic haematopoietic stem cell transplantation.

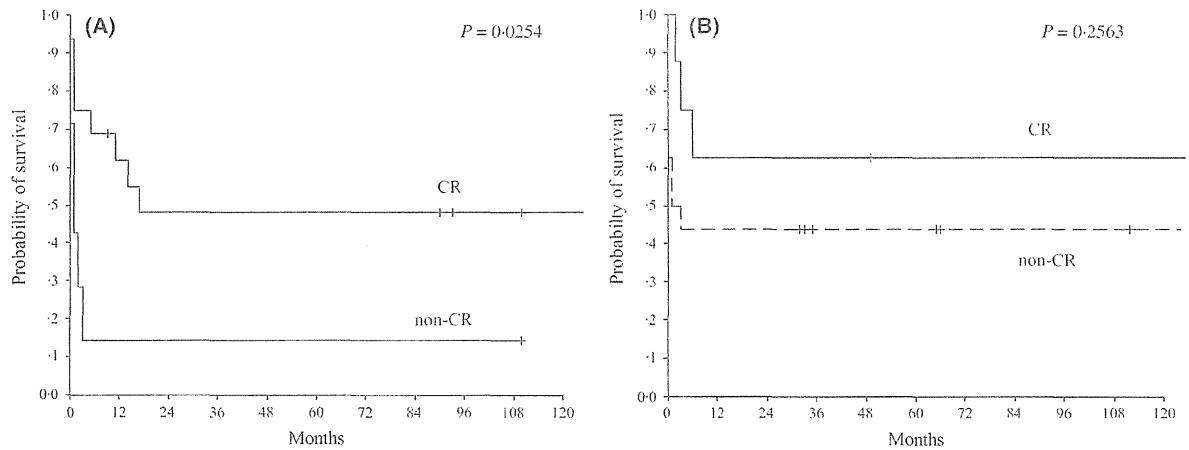

Fig 3. Event-free survival according to disease status at HSCT. (A) Autologous HSCT, (B) allogeneic HSCT. HSCT, haematopoietic stem cell transplantation; CR complete remission.

Table II. Details and outcomes of patients treated with reduced intensity conditioning and allogeneic HSCT.

Patients	Status at HSCT	Age at HSCT (years)	Donor	Stem cell source	Conditioning regimen	GVHD prophylaxis	aGVHD (Grade)	Extensive cGVHD	Outcome	Follow-up (months)
1	PR	3	UD	CB	TLI 2 Gy, Flu, Mel	Tac, MTX	III	–	CR	32
2	PR	9	UD	CB	Flu, Mel	Tac, MTX	II	–	CR	65
3	CR	18	UD	BM	Flu, Mel, ATG	Tac, MTX	0	NA	TRM	5
4	PR	16	UD	BM	Bu, Flu	Tac, MTX	III	–	CR	33

HSCT, haematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; UD, unrelated donor; BM, bone marrow; CB, cord blood; TLI, total lymphoid irradiation; Bu, busulfan; Flu, fludarabine; Mel, melphalan; ATG, antithymocyte globulin; GVHD, graft-*versus*-host disease; Tac, tacrolimus; MTX, methotrexate; aGVHD, acute GVHD; cGVHD, chronic GVHD; TRM, treatment-related mortality; NA, not applicable.

had predictive value for OS and EFS. In the current study, the EFS of the patients with CR at autologous HSCT was significantly higher than that of the patients with non-CR at autologous HSCT. Brugières *et al* (2000) reported that an interval of <12 months between diagnosis and relapse was associated with a higher risk of failure for the treatment of relapsed ALCL, including autologous HSCT. However, our cohort did not provide sufficient data to compare the risk of failure with the interval between diagnosis and relapse.

The role of allogeneic HSCT has not been defined for cases of childhood ALCL. The currently available evidence is limited to a few reports. The BFM group reported a series of 20 paediatric patients who underwent allogeneic HSCT for relapsed or refractory ALCL, finding a 75% 3-year EFS (Woessmann *et al*, 2006). Twelve of the patients in this study were in CR at HSCT. The CIBMTR has reported another large series of allogeneic HSCTs that were performed for ALCL, observing an EFS of 46% for 12 relapsed or refractory patients (Gross *et al*, 2010). Giulino-Roth *et al* (2013) also reported the cases of 13 paediatric patients with ALCL, eight of whom underwent autologous HSCT and five of whom underwent allogeneic HSCT. The OS and disease-free survival rates were 83% and 77%, respectively. Although our previous study noted that all six patients who underwent allogeneic HSCT during their second CR survived without further relapse (Mori *et al*, 2006), 5-year OS and EFS rates were limited to 54% and 50% in the present study. Patients who underwent allogeneic HSCT while in CR accounted for only eight of the 24 cases. Indeed, the rate of CR at HSCT was lower in the current study than in previous reports of allogeneic HSCT. In the present study, we found no significant difference in EFS according to disease status (CR or non-CR) at allogeneic HSCT. However, the low CR rate at allogeneic HSCT might be associated with the survival rate in the current study, which was lower than the rates noted in previous reports.

In the present study, we observed a 25% TRM rate among patients who underwent allogeneic HSCT for relapsed and refractory disease. Although the cumulative incidence of TRM for allogeneic HSCT was higher than that for autologous HSCT, the difference was not significant ($P = 0{\cdot}40$) (Fig 1B). Several investigations have shown that RIC followed by allogeneic HSCT has the potential to reduce TRM and long-term toxicity in cases of malignant and non-malignant diseases (Carella *et al*, 2000; Dreger *et al*, 2003; Jacobsohn *et al*, 2004; Bradley *et al*, 2007). The BFM cohort of allogeneic HSCTs included one case in which an RIC regimen was administered to a patient with ALCL. The RIC regimen comprised total lymphoid irradiation (2 Gy), fludarabine and melphalan (Brugières *et al*, 2000). Another case in which an RIC regimen [thraco-abdominal irradiation (2 Gy), fludarabine and melphalan] was used has also been reported (Ohta *et al*, 2010). Both of these patients survived in continuous CR following allogeneic HSCT. In the present study, four patients received an RIC regimen followed by allogeneic HSCT. Of these four patients, three were in non-CR at allogeneic HSCT, yet survived in CR for 32–65 months without relapse after HSCT. These results suggest that RIC for relapsed or refractory ALCL may be useful in cases involving allogeneic HSCT, regardless of disease status. However, there are only a few reports of allogeneic HSCT using an RIC regimen for paediatric ALCL. Further evaluations of the efficacy of RIC are necessary and should include larger numbers of patients and a prospective design.

The treatment of relapsed or refractory ALCL remains a matter of debate. Recent studies have reported the efficacies of second-line treatments for relapsed or refractory ALCL, including vinblastine monotherapy, brentuximab vedotin and crizotinib. Brugières *et al* (2009b) studied 36 paediatric patients treated with weekly vinblastine for relapsed or refractory ALCL, finding that this treatment was highly efficacious, with a CR rate of 83%. Furthermore, the 5-year EFS rate was 30%, at which time all but two of the patients had stopped vinblastine for more than 2 years. In adults, a phase II trial of brentuximab vedotin was conducted in patients with relapsed or refractory systemic ALCL. Fifty of 58 patients (86%) achieved an objective response, including 33 patients (57%) in CR (Pro *et al*, 2012). The Children's Oncology Group reported a phase I study of crizotinib for paediatric patients with refractory ALCL, finding that seven of nine children acheived CR following crizotinib monotherapy (Mossé *et al*, 2013). Autologous and allogeneic HSCTs are associated with high rates of toxicities and TRM. Consequently, it will be necessary to speculate about the selection of second-line treatments for relapsed or refractory ALCL in children and adolescents.

In conclusion, both autologous and allogeneic HSCT can offer the prospect of durable disease-free survival for relapsed and refractory ALCL in childhood and adolescence. Patients with CR at the time of autologous HSCT had significantly greater EFS than patients with non-CR at the time of autologous HSCT. Our results suggest that allogeneic HSCT might provide a better outcome for patients who are resistant to chemotherapy after relapse, and those with non-CR at the time of HSCT. Furthermore, an RIC regimen followed by allogeneic HSCT might even be useful for these patients. However, the small number of patients in our cohort prevented us from investigating the efficacy of allogeneic HSCT with an RIC regimen. In the new era of molecular target drugs, the best candidates for autologous and allogeneic HSCT remain to be clarified by further analyses and prospective studies of relapsed or refractory ALCL in childhood and adolescence.

Acknowledgements

We thank each of the clinicians, hospital administrators and health centre administrators who provided precise data via the registry of the Japan Society for Stem Cell Transplantation.

Author contributions

R Kobayashi, T Mori and R Fukano designed the research study; M Chin, H Goto, Y Takahashi, J Hara, YD Park, M Inoue, Y Koga, J Inagaki, H Sakamaki, S Adachi, K Kawa, K Kato and R Suzuki collected the data; R Fukano analysed the data and wrote the paper. All authors reviewed the manuscript.

Conflict of interest

There are no conflicts of interest to declare.

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Biol Blood Marrow Transplant xxx (2014) 1–7

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Choreito Formula for BK Virus–associated Hemorrhagic Cystitis after Allogeneic Hematopoietic Stem Cell Transplantation

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Article history:
Received 28 August 2014
Accepted 21 October 2014

Key Words:
BK virus
Hemorrhagic cystitis
Pediatric
Choreito
Kampo medicine

A B S T R A C T

Therapy for BK virus (BKV)–associated hemorrhagic cystitis (BKV-HC) is limited after hematopoietic stem cell transplantation (HSCT). We examined whether choreito, a formula from Japanese traditional Kampo medicine, is effective for treating BKV-HC. Among children who underwent allogeneic HSCT between October 2006 and March 2014, 14 were diagnosed with BKV-HC (median, 36 days; range, 14 to 330 days) after HSCT, and 6 consecutive children received pharmaceutical-grade choreito extract granules. The hematuria grade before treatment was significantly higher in the choreito group than in the nonchoreito group ($P = .018$). The duration from therapy to complete resolution was significantly shorter in the choreito group (median, 9 days; range, 4 to 17 days) than in the nonchoreito group (median, 17 days; range, 15 to 66 days; $P = .037$). In 11 children with macroscopic hematuria, the duration from treatment to resolution of macroscopic hematuria was significantly shorter in the choreito group than in the nonchoreito group (median, 2 days versus 11 days; $P = .0043$). The BKV load in urine was significantly decreased 1 month after choreito administration. No adverse effects related to choreito administration were observed. Choreito may be a safe and considerably promising therapy for the hemostasis of BKV-HC after HSCT.

INTRODUCTION

Hemorrhagic cystitis (HC) is a severe complication in patients undergoing hematopoietic stem cell transplantation (HSCT), resulting in significant morbidity, such as nephropathy and renal failure, prolonged hospitalization, and prolonged blood transfusion requirement [1,2]. Effects on mortality have also been reported in children undergoing HSCT [3]. Early-onset HC occurs within 1 week after HSCT and is mostly a symptom of regimen-related toxicity. Late-onset HC usually occurs after engraftment and is associated with viral infections, including those caused by the human polyomavirus BK (BKV), polyomavirus JC, adenovirus (AdV), and cytomegalovirus (CMV) [4]. BKV is the most frequent cause of late-onset HC and affects 5.3% to 21.2% of children undergoing HSCT [5-9]. BKV viruria is detected by real-time quantitative PCR (RT-PCR) in all patients with BKV-HC. A BKV load of more than 10^6 copies/mL in urine may be associated with a high risk of developing HC after HSCT [5]. However, asymptomatic BK viruria is detected in 50% to 100% of patients after HSCT [5,7,10], implicating that the presence of BKV viruria alone does not explain the pathogenesis of HC. High BKV viremia ($\geq 10^3$ copies/mL) is a better predictor of BKV-HC after HSCT, with a reported specificity of 93% [8]. Children with high BKV viremia ($\geq 10^4$ copies/mL) are at a higher risk of developing severe HC [6].

The standard treatment for BKV-HC has not been established [2]. Supportive therapy is provided to patients with mild BKV-HC, including intravenous hydration, bladder irrigation, and symptomatic relief treatment, such as the use of analgesics. Patients with severe BKV-HC require additional therapy. The current first line BKV-oriented therapy is intravenous cidofovir; however, its efficacy remains controversial [2]. Alternative strategies include intravesical instillation of cidofovir [2,7], hyperbaric oxygen therapy [11], leflunomide, and fluoroquinolone [12]; however, their effect is limited [13]. Invasive intervention such as vascular embolization or cystectomy may be necessary in uncontrollable HC.

Choreito is a formula derived from Japanese traditional Kampo medicine. The indication for choreito in the context of

Financial disclosure: See Acknowledgments on page 6.

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http://dx.doi.org/10.1016/j.bbmt.2014.10.018
1083-8791/

Kampo medicine is "dampness-heat" in the lower abdomen, the characteristic symptoms of which include dysuria, heat in the lower abdomen, and thirst. All these symptoms may be caused by inflammation and blood clots in the bladder. Based on this indication, choreito has been administered to patients with acute simple cystitis and urolithiasis, and its effectiveness has been confirmed [14]. Recently, choreito was successfully used to treat massive gross hematuria with clot retention in the bladder in a child with refractory acute lymphoblastic leukemia [14]. At present, choreito is covered by the national health insurance and is widely used for genitourinary symptoms in Japan.

Symptoms leading to the traditional use of choreito appear to overlap with symptoms associated with BKV-HC; indeed, some children receive choreito for HC. In this study, we retrospectively analyzed BKV-HC in children undergoing HSCT and evaluated the efficacy of choreito treatment.

PATIENTS AND METHODS

Definition

HC was defined as microscopic (blood in urine graded 1+ or more) or macroscopic hematuria combined with dysuria, pollakisuria, urinary urgency, and/or the sensation of residual urine in the absence of bacteria in urine as observed by culture [9]. *BKV-HC* was defined as the association of HC with BKV viruria and/or viremia. HC was graded according to the widely used criteria [15]. Grade I is defined as microscopic hematuria, grade II as macrohematuria, grade III as macroscopic hematuria with clots, and grade IV as macroscopic hematuria with renal or bladder dysfunction. The onset of BKV-HC was defined as the first day when patients presented with urinary symptoms, and *complete resolution* (CR) of HC was defined as blood in urine (− or + for hemoglobin) and disappearance of dysuria, pollakisuria, urinary urgency, and the sensation of residual urine related to HC.

Patient Inclusion Criteria of BKV-HC and Choreito Administration

Among the children (≤18 years old) who received allogeneic HSCT between October 2006 and March 2014 in Nagoya University Hospital, 14 were diagnosed with BKV-HC and included in the study. Their medical records were retrospectively analyzed. Patient characteristics are listed in Table 1. Intravenous fluids corresponding to 2.5 to 3.0 L/m^2/day with forced alkalinized diuresis were administered during conditioning, and patients treated with cyclophosphamide received prophylactic mesna for the prevention of HC. All the patients received acyclovir for herpes prophylaxis and weekly intravenous immunoglobulin for viral prophylaxis. Tacrolimus was intravenously administered for graft-versus-host disease (GVHD) prophylaxis in patients receiving HSCT from an unrelated donor. Cases of engraftment syndrome and GVHD were treated by methylprednisolone, followed by salvage therapies in nonresponding patients. Six children with BKV-HC diagnosed after March 2013 received a pharmaceutical-grade medicine, choreito extract granules (Tsumura & Co., Tokyo, Japan) with a dose of .2 g/kg per os daily in 3 divided doses (maximum, 7.5 g/day). Cidofovir and choreito were administered at the onset of macroscopic hematuria. Because it is not currently approved for clinical use in Japan, cidofovir was administered only to those who provided written informed consent.

Quantification of BKV DNA

Children undergoing HSCT were weekly monitored for plasma CMV, human herpesvirus 6, and Epstein-Barr virus, and those who met the criteria for HC underwent additional viral workup, including analysis for BKV, polyomavirus JC, and AdV. For 2 patients with BKV diagnosed before December 2009, BKV had been detected in urine by qualitative PCR. This qualitative PCR could not detect BKV in patients without HC. After January 2010, viruses were monitored by multiplex RT-PCR for quantification of DNA from BKV, polyomavirus JC, and AdV, as described previously [16]. In April 2010, BKV RT-PCR was used to screen all 30 hospitalized children with various hemato-oncological diseases who had neither HC-related symptoms nor abnormal urinalysis. All patients provided informed consent for viral PCR workup in accordance with the Declaration of Helsinki. This retrospective analysis was approved by the ethics committee of Nagoya University Graduate School of Medicine.

Statistical Analysis

Statistical analysis was performed using the Fisher's exact test for categorical variables and the Mann-Whitney's U test for continuous variables. The Wilcoxon signed-rank test was used for paired samples. Odds ratios with confidence intervals were estimated by the logistic regression. A probability (P) value <.05 was considered to indicate statistical significance. All statistical analyses were conducted using JMP Pro 11.0.0 (SAS Institute Inc., Cary, NC).

RESULTS

BKV Screening in Hemato-oncological Patients without Genitourinary Symptoms

All children with hemato-oncological disorders hospitalized in the same ward were screened for BKV viruria for the purpose of surveillance. BKV viruria was detected in 5 (17%) of 30 hospitalized children with various hemato-oncological diseases who had neither HC-related symptoms nor abnormal urinalysis. The median urine BKV load in children with asymptomatic viruria was 1.3×10^6 copies/mL (range, 3.5×10^3 to 2.0×10^9 copies/mL), which was significantly lower than that in children with BKV-HC (median, 5.4×10^{10} copies/mL; range, 8.3×10^7 to 1.5×10^{11} copies/mL; $P = .0021$).

Patient Characteristics of Cases with BKV-HC after HSCT

Table 1 summarizes the patient characteristics of 14 children who underwent HSCT and later developed BKV-HC. In patients 1 and 2, BKV was detected in urine by qualitative

Table 1
Patient Demographics of BKV-HC after HSCT

UPN	Choreito Treatment	Age, yr	Sex	Diagnosis	Clinical Status	Preconditioning Regimen	Stem Cell Source	GVHD Prophylaxis
1	No	15.3	M	AA	Non CR	CY + ATG + TBI 5 Gy	UR-BM	FK + sMTX
2	No	16.0	M	AA	Non CR	FLU + CY + Campath + TBI 3 Gy	UR-BM	FK + sMTX
3	No	12.3	M	B-ALL	CR1	MEL + TBI 12 Gy	UR-BM	FK + sMTX
4	No	11.8	M	CML	CyCR	FLU + MEL + TBI 3 Gy	UR-BM	FK + sMTX
5	No	7.1	F	T-ALL	CR2	FLU + MEL + ATG + TBI 12 Gy	Haplo	FK + sMTX
6	No	5.7	M	NB	CR1	FLU + MEL + TBI 2 Gy	UR-CB	FK + sMTX
7	No	15.4	M	CMML	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
8	No	7.8	M	B-ALL	CR2	MEL + ATG + TBI 12 Gy	UR-BM	FK + sMTX
9	Yes	14.3	M	AA	Non CR	FLU + MEL + ATG + TBI 3 Gy	Haplo	FK + sMTX
10	Yes	5.4	M	MDS	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
11	Yes	10.1	F	AA	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
12	Yes	12.2	F	CMML	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
13	Yes	6.8	M	B-ALL	CR2	MEL + TBI 12 Gy	UR-BM	FK + sMTX
14	Yes	7.5	M	MDS	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX

UPN indicates unique patient number; M, male; AA, aplastic anemia; Cy, cyclophosphamide; ATG, antithymocyte globulin; TBI, total body irradiation; UR, unrelated; BM, bone marrow; FK, tacrolimus; sMTX, short course of methotrexate; FLU, fludarabine; Campath, alemtuzumab; ALL, acute lymphoblastic leukemia; MEL, melphalan; CML, chronic myelogenous leukemia; CyCR, cytological complete remission; F, female; Haplo, haploidentical transplant; NB, neuroblastoma; CB, cord blood; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome.

PCR; therefore, other agents including preconditioning could have contributed to HC. Six of the 14 children received choreito because of BKV-HC. All patients were older than 5 years (median, 11 years; range, 5.4 to 16 years). Antithymoglobulin or alemtuzumab was administered to 10 of 14 children (71%) as a preconditioning. Notably, all the children received total body irradiation with various doses.

Children were diagnosed with BKV-HC at a median 36 days (range, 14 to 330 days) (Table 2) after HSCT. Six of 14 patients (43%) had grade II to IV acute GVHD, and 11 of 14 (79%) received steroids for treatment of engraftment syndrome and/or acute GVHD before being diagnosed with BKV-HC. Three children with acute GVHD grade III or IV received intensified immunosuppressive treatment for steroid-resistant GVHD; 1 received infliximab and the other 2 received infliximab, basiliximab, and mesenchymal stem cells. All 3 responded well to additional therapy for acute GVHD. Concomitant AdV viruria was detected in 2 of 14 children (14%), and 12 of 14 children (86%) developed CMV and/or Epstein-Barr virus infection after HSCT. AdV titers in the urine were 2.6×10^8 copies/mL in patient 3 and 1.8×10^8 copies/mL in patient 7 at the time of diagnosis. CMV viruria was not detected in any of these 14 children when BKV-HC was diagnosed. Six children were receiving gancyclovir and/or foscarnet for CMV reactivation at the time of BKV-HC diagnosis.

Treatment for BKV Cystitis with Choreito

Six of 14 children with BKV-HC diagnosed after October 2013 received choreito (Tables 1 to 3). All 6 fulfilled the Kampo indication for receiving choreito ("lower energizer dampness-heat" in patients 9, 11, 12, 13, and 14, and "heat binding in the lower energizer" in patient 10). Patient characteristics, including age at HSCT, sex, underlying disease, engraftment syndrome, acute GVHD frequency and grade, immunosuppressive treatment, absolute lymphocyte count, antiviral therapy, duration of steroid use before the diagnosis of BKV-HC, and duration from HSCT to the onset of BKV-HC, did not differ significantly between the choreito group and the nonchoreito group (Tables 1 and 2). However, the hematuria grade at the time of diagnosis of BKV-HC was significantly higher in the choreito group than in the nonchoreito group ($P = .018$) (Table 2). Choreito was administered over a median of 5 days after the onset of symptoms related to BKV-HC (range, 2 to 16 days), and this interval was not statistically different from that of other treatments (median, 4 days; range, 1 to 23 days; $P = .43$) (Table 3). The urine BKV load before treatment amounted to a median of 2.6×10^{10} copies/mL (range, 1.3×10^9 to 6.3×10^{10} copies/mL) in children receiving choreito, which was not statistically different from that in those not receiving choreito (median, 3.4×10^{10} copies/mL; range, 8.3×10^7 to 1.3×10^{11} copies/mL; $P = .67$) (Table 3). Similarly, the BKV load in whole blood before treatment was not statistically different between the choreito and nonchoreito groups ($P = .24$, Table 3).

In all 14 children with BKV-HC, the duration from the start of therapy to CR as defined by disappearance of dysuria, pollakisuria, urinary urgency, and the sensation of residual urine was significantly shorter in the choreito group (median, 9 days; range, 4 to 17 days) than in the nonchoreito group (median, 17 days; range, 15 to 66 days; $P = .037$) (Table 3, Figure 1A): the odds ratio of choreito versus nonchoreito was .63 (95% confidence interval, .22 to .93; $P = .0031$). With regard to 11 children with HC graded ≥ II at the beginning of therapy, the administration of choreito

Table 2
Clinical Characteristics of Patients with BKV Cystitis

UPN	Engraftment Syndrome	Acute GVHD		ALC at the Diagnosis of BKV-HC ($\times 10^9$/L)	Steroid Use (d before BKV-HC)	Other Immunosuppressants	Onset of BKV-HC (d from SCT)	Hematuria (Grade)	Viruria (Urine log copy/mL)	CMV (Whole Blood log copy/mL)	Viral Infections	Antiviral Therapy at BKV-HC
		Stage	Grade									
1	+	-	-	4.7	14	-	35	II	BKV	0.0	CMV, EBV	GCV
2	-	skin 3	II	.3	24	-	65	II	BKV	3.1	CMV	PFA
3	+	-	-	.8	10	-	36	III	BKV (9.2), AdV (8.4)	0.0	CMV	PFA
4	+	skin 3	II	1	90	-	330	II	BKV (7.9)	0.0	CMV, EBV	PFA
5	+	skin 2, gut 1	II	.2	-	-	14	II	BKV (10.8)	2.6	CMV	-
6	-	skin 2, gut 3	III	1	10	INX	45	I	BKV (10.9)	0.0	-	-
7	-	skin 3, gut 2	III	.6	67	INX, BSX, MSC	86	I	BKV (11.1), AdV (8.3)	3.0	CMV, EBV	-
8	+	-	-	2	2	-	27	II	BKV (10.0)	3.2	CMV	-
9	-	-	-	.2	-	-	16	III	BKV (9.1)	2.9	EBV	-
10	+	skin 2, liver 4, gut 2	IV	.8	12	INX, BSX, MSC	25	III	BKV (9.2)	0.0	-	-
11	+	-	-	1.8	30	-	48	III	BKV (9.5)	0.0	CMV, EBV	GCV + PFA
12	+	-	-	.2	45	-	67	III	BKV (10.8)	2.7	CMV	GCV
13	-	-	-	1.3	-	-	21	III	BKV (10.7)	0.0	CMV	-
14	+	-	-	.5	6	-	26	I	BKV (10.7)	0.0	EBV	-

ALC indicates absolute lymphocyte count; SCT, stem cell transplanation; EBV, Epstein-Barr virus; GCV, gancyclovir; PFA, foscarnet; INX, infliximab; BSX, basiliximab; MSC, mesenchymal stem cell transplantation.

Table 3
Summary of Treatment for Patients with BKV Cystitis

UPN	Duration from Onset to Tx, d	Primary Tx for BKV	Hematuria Grade at Tx	Hematuria Grade ≤I (d from Tx)	CR (d from Tx)	Urine BKV Load before Tx (log copy/mL)	Plasma BKV Load before Tx (log copy/mL)	Urine BKV Load 1 mo after Tx (log copy/mL)	Plasma BKV Load 1 mo after Tx (log copy/mL)	Possible Complications
1	7	Cidofovir (5 mg/kg qwk ×2), hydration	II	11	17	N/A	N/A	N/A	N/A	None
2	4	Bladder irrigation, hydration	II	16	55	N/A	N/A	N/A	N/A	None
3	14	Cidofovir (1 mg/kg qwk ×2), hydration	III	28	66	9.2	0.0	6.5	3.8	Renal failure
4	4	Hydration	II	10	15	7.9	0.0	N/A	N/A	None
5	2	Hydration	II	5	16	10.8	0.0	N/A	N/A	None
6	1	Hydration	I	N/A	15	10.9	3.0	10.5	3.6	None
7	1	Hydration	I	N/A	15	11.1	0.0	N/A	N/A	None
8	23	Hydration	II	8	23	10.0	0.0	N/A	N/A	None
9	16	Choreito, cidofovir (1 mg/kg qwk ×11), hydration	III	4	6	9.1	4.0	8.7	4.6	None
10	5	Choreito	III	2	4	9.2	3.1	8.3	4.0	None
11	2	Choreito	III	2	16	9.5	0.0	7.8	0.0	None
12	4	Choreito	III	3	17	10.8	0.0	8.2	5.8	None
13	5	Choreito	III	2	7	10.7	5.0	4.4	0.0	None
14	16	Choreito	I	N/A	11	10.7	2.1	10.5	3.2	None

Tx indicates treatment; qwk, every week; N/A, not applicable or available.

significantly shortened the duration from the onset to BKV-HC grade ≤ I (median, 2 days; range, 2 to 4 days) in comparison with that in the nonchoreito group (median, 11 days; range, 5 to 28 days; $P = .0043$) (Table 3, Figure 1B). The duration from start of therapy to CR was also significantly shorter in the choreito group (median, 7 days; range, 4 to 17 days) than in the nonchoreito group (median, 20 days; range, 15 to 66 days; $P = .048$) (Table 3, Figure 1C): here, the odds ratio of choreito versus nonchoreito was .66 (95% confidence interval, .14 to .95; $P = .0058$).

Sequential Analysis of BKV Load after Choreito Treatment

BKV-HC–related symptoms improved significantly earlier in children receiving choreito, and we studied whether these earlier improvements were related to the clearance of BKV. The BKV load in urine and whole blood was monitored after the diagnosis of BKV-HC in children receiving choreito. The urine BKV load generally decreased over time. The median urine BKV load was 1.7×10^8 copies/mL (range, 2.6×10^4 to 3.1×10^{10} copies/mL) 1 month after BKV-HC diagnosis when all children had achieved CR, and they experienced a statistically significant decrease in BKV load since the time of diagnosis ($P = .031$; Wilcoxon signed-rank test for paired samples) (Table 3, Figure 2A). At the time of CR, only 1 of 6 children had a urine BKV load lower than 1.3×10^6 copies/mL, which was the median urine BKV load in children with asymptomatic viruria. The BKV load in whole blood appeared stable during the course of BKV-HC, and no significant decrease was observed a month after diagnosis ($P = .44$) (Table 3, Figure 2B).

All 6 children eventually finished taking choreito, and relapse of HC was not observed, except for in 1 patient who experienced relapse twice (patient 9). This patient was diagnosed with idiopathic aplastic anemia and received a bone marrow transplant from an unrelated donor; however, the graft was rejected and he underwent haplo-identical HSCT as the second HSCT. Because he developed chronic GVHD, he was administered prednisolone, which was increased during the exacerbation of chronic GVHD and which may have contributed to the prolonged elevation of the BKV load. Every time the patient had a relapse of BKV-HC, he was administered choreito, and his genitourinary symptoms resolved within a few days (Supplemental Figure 1).

Safety and Tolerability of Treatment

All children were able to take choreito per os. Notably, there were no adverse effects due to choreito intake, and renal function impairment was not observed in children receiving choreito (Table 3). The reported adverse effects of choreito include drug allergy and mild gastric discomfort [14], which were not observed in any of the children. In the nonchoreito group, 1 patient (patient 3) who received cidofovir for BKV infection developed impaired renal function, possibly resulting from renal toxicity of cidofovir and post-renal acute kidney injury due to clot retention.

DISCUSSION

Unlike its effect in immunocompetent patients, HC is life threatening in immunocompromised patients with hemato-oncological disease, particularly among patients undergoing HSCT [17]. To our knowledge, prospective studies of the treatment for BKV-HC are not available, and there are no standard treatment guidelines for post-HSCT HC. Treatment modalities are limited, particularly in children, partly owing to few reports on children receiving pharmaceutical and

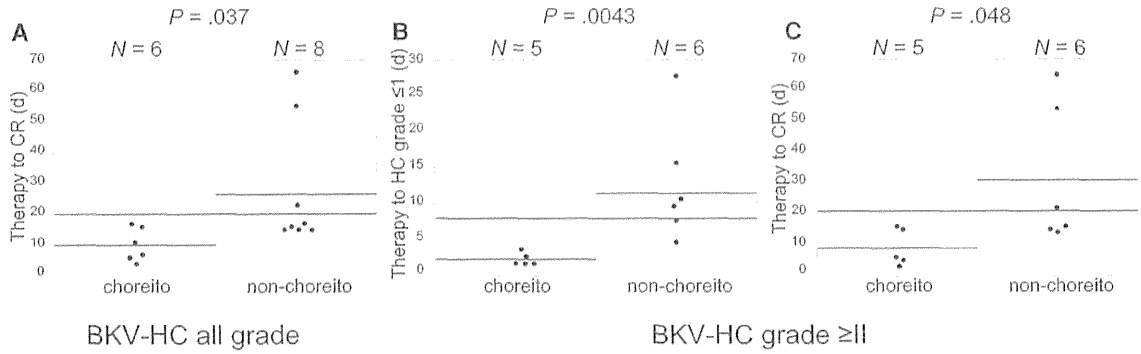

Figure 1. Comparison of choreito and nonchoreito treatment for BK virus-associated hemorrhagic cystitis (BKV-HC). The duration from the beginning of therapy to complete resolution (CR), as defined by the absence of dysuria, pollakisuria, urinary urgency, or the sensation of residual urine, was shorter in the choreito group (median, 9 days; range, 4 to 17 days) than in the nonchoreito group (median, 17 days; range, 15 to 66 days; $P = .037$) (A). When comparing children with HC graded ≥ II, the administration of choreito significantly shortened the duration from the onset to BKV-HC grade ≤ I (median, 2 days; range, 2 to 4 days) in comparison with that in the nonchoreito group (median, 11 days; range, 5 to 28 days) (B). The duration from start of therapy to CR was also significantly shorter in the choreito group (median, 7 days; range, 4 to 17 days) than in the nonchoreito group (median, 20 days; range, 15 to 66 days; $P = .048$) (C).

surgical treatments [4,18-20]. Intravenous hydration with forced diuresis is conducted; however, this is supportive treatment only without reliable efficacy.

At present, cidofovir is the only commercially available antiviral agent against BKV, and its efficacy for BKV-HC has been investigated only in retrospective studies [19-21]. In the report from the European Group for Blood and Marrow Transplantation, intravenous or intravesical cidofovir was administered to 62 patients with BKV-HC [21]. Of the 62 patients, 41 (66%) achieved CR and 8 (13%) had partial response after cidofovir treatment; however, no improvement or deterioration was observed in 12 patients (19%). CR is related to clearance of BK viremia in patients with BK viremia detected at the beginning of treatment, and the median time to clearance is 37 days (range, 7 to 102 days). Of 57 patients receiving intravenous cidofovir, 17 (30%) experienced renal toxicity. In a pediatric cohort, 19 children received cidofovir for BKV-HC grade ≥ II [19]. Macroscopic hematuria resolved in 15 (79%) after a median of 22 days (range, 9 to 63 days). In 1 patient, HC progressed to grade IV during cidofovir treatment. Notably, the baseline creatinine level appeared to be elevated after treatment. Another pediatric cohort included 12 children with BKV-HC treated by intravenous and/or intravesical cidofovir [20]. The median duration of symptoms was 25 days (range, 9 to 73 days) and no persistent nephrotoxicity was observed. Compared with cidofovir treatment, children treated with choreito treatment in our study experienced no impairment of renal function; all patients with BKV-HC achieved CR and BKV-HC resolved earlier.

Hyperbaric oxygen therapy is another alternative treatment for BKV-HC [11,22]. A retrospective study included 16 patients with BKV-HC grade ≥ II (5 patients under 19 years of age), 15 (94%) of whom achieved CR after a median of 17 days (range, 4 to 116 days) [11]. In a pediatric cohort of 10 children with BKV-HC grade ≥ II, 9 (90%) achieved CR after a median of 15 days (range, 10 to 37 days), including spontaneous resolution [22]. Hyperbaric oxygen is generally well tolerated; however, it requires a high-cost facility and adverse effects have been reported, including ruptured tympanum.

Other alternative therapies include leflunomide and fluoroquinolone antibiotics [12]; however, experience is limited, even in adults [13]. Few reports of leflunomide use in the setting of HSCT are available and its safety has not been

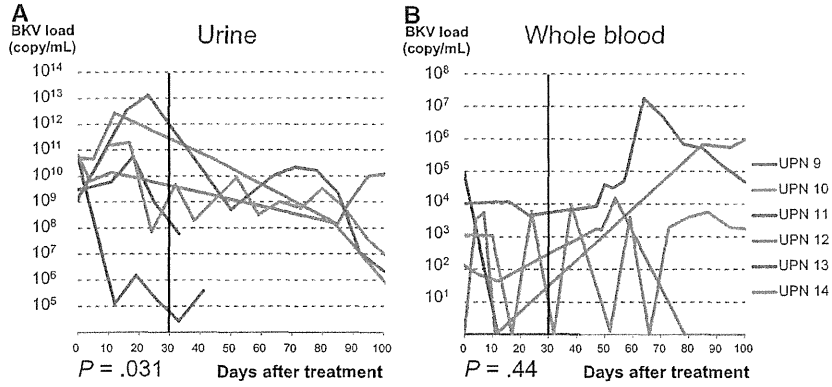

Figure 2. BK virus (BKV) load after choreito treatment. The BKV load before treatment amounted to a median of 2.6×10^{10} copies/mL in urine (range, 1.3×10^9 to 6.3×10^{10} copies/mL) and a median of 6.5×10^2 copies/mL in whole blood (range, 0 to 9.0×10^4 copies/mL). The median urine BKV load was 1.7×10^8 copies/mL (range, 2.6×10^4 to 3.1×10^{10} copies/mL) 1 month after BKV-HC diagnosis, and the BKV load had significantly decreased since the time of diagnosis (Wilcoxon signed-rank test, $P = .031$) (A). The BKV load in whole blood appeared stable during the course of BKV-HC, and no significant decrease was observed a month after diagnosis (Wilcoxon signed-rank test, $P = .44$) (B).

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